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Study of the Biodeterioration of some Stained Glasses by the Fungus *Stemphylium botryosum*

[1]Maisa M.A. Mansour, [2]Nagwa S. Abdel-Rahim and [3]Mohamed Z.M. Salem

[1]*Conservation Department, Faculty of Archaeology, Cairo University, Giza, 12613, Egypt.*
[2]*Conservation Department, Faculty of Archaeology, Fayoum University, Fayoum, Egypt.*
[3]*Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt.*

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ABSTRACT

Stained glasses are widely used in windows of the buildings and exposed for many different damage factors that affect the composition and properties such as humidity, temperature, air pollution and microorganisms and other factors of the damage. The present study investigated the biodeterioration states of the surface of six colored glasses- which used in stained glass- (black dark purple, opaque white, dark green, black dark, yellow semitransparent and red oxide cuprite) by the fungus *Stemphylium botryosum* after incubation for 6 months. Scanning electron microscopy attached with energy dispersive spectrometry (ESM-EDAX) was used to investigate the growth of *S. botryosum* on the glass sample composition before and after infection. Also the chromatic alternation was measured according to CIELAB system. Results showed that S. *botryosum* has developed clear changes on all six colored glasses surface as an alternation in element oxides, and the formation of hyphal. The result of glass decay is a sharp decrease of the network composition (SiO_2) as well as increase in CO_2. We have shown that *S. botryosum* can colonize various types of glasses where the chemical elements necessary to its development become available. Dark green and black dark purple were the most affected colored glasses by *S. botryosum* with SiO_2 was decreased from 64.13% to15.41% and 62.97% to 30.52%, respectively. The results revealed that, samples treated with *S. botryosum* have been decreased the oxide Cu in the Dark green from 0.15% to 0.11%and Mn in black dark purple from 0.16 %to 0.05. Therefore, *S. botryosum* was able to biodeteriorate the colored glasses with distinct compositions. (Regarding the biodeterioration degree, there were differences between all the colored glasses in terms of alternation in element oxides compositions).

Key words: Colored glasses; *Stemphylium botryosum*; SEM-EDAX; Chemical composition; Biodeterioration

Introduction

Glass damage occurs due to the action of microbes, but there are a complicated relationship between glass damage and other factors like; glass composition, chemical durability of glass, effects of environmental and human factors and biogenic action, making precise statements concerning the corrosion mechanisms difficult (Drewello and Weissmann, 1997).

Microbes play role in all environments. Microbes are found in air, water, and soil and their impact on material, such as general environmental public health, microbiology, microbial ecology, and biodegradation and biotransformation. (Fry and Evans, 1977). Three isolates of *Stemphylium botryosum Wallr* produced the toxin on different liquid cultures about 95% of stemphol was found in the mycelium, and toxin production was slightly increased when cultures were exposed to near UV light rather than being grown in the dark (Solfrizzo *et al.,* 1994). The stained-glass windows exposed to the open air, sun rays directly whish containing Ultra Violet light.

Stained glass is made up of network formers, stabilizers, modifiers, and coloring elements (Römich, 1999) and several metals, such as Cu, Co, and Mn, were used to color the glass (Bamford, 1977; Werner, 1978; Newton and Davison, 1989). Stained glass is colored by metallic oxide additives (*i.e.,* CuO, Na_2O, Al_2O_3, Fe_2O_3, and P_2O_5) and the glass panels can then be painted, enameled, stained, etched, or engraved (Newton and Davison, 1989). The glass colors were also depends on many factors such as the purity of the raw materials and the melting temperature of treatment and to oxidation or reduction atmosphere furnace (Frank, 1982). Also cobalt (Co) was used to obtain the dark (Neuburg, 1962) or black (Nicholson, 1993) color for glass.

Glass color depends upon the result of using of Co on the type and installation of glass and the degree of equality of the oxide as the blue color results from the presence of the ion bilateral CO^{+2}, which enters in the

Corresponding Author: Nagwa S. Abdel-Rahim, Conservation Department, Faculty of Archaeology, Fayoum University, Fayoum, Egypt.
E-mail: nsa00@fayoum.edu.eg

networked structure of the glass as a component, where the connection with other ions through four O atoms until this installation up to the case that electrically neutral (Bacci and Picollo, 1996). But there must be a high temperature and high alkaline have found that Co is used in the form of $CoAl_2O_4$. Also $CoAl_2O_4$ was used as a tinted glass and have found that the increase in alkaline ratio increases the puddle color as they reach the proportion of alkali glass between 16%-21% (Na_2O), while calcium oxide ratio of up to about 4.9% of the total aluminum ratio of about 1.6 to 2% and up in the glass Co ratio of about 3, 5% of glass components (Tite, 2000). Generally, it also contains more Al_2 and less in K and this is characterized by this type of glass Co from other glass used other colors (Rehren, 2000). For stained glasses, Rousseau (2010) reported that silver-yellow was commonly used as a stain, while opaque, dark brown or black iron oxide glass paint (grisaille) was used to outline forms and create shading.

The biodeterioration of glass was firstly reported in the beginning of past century (Mellor, 1924), however, with the wrong environmental conditions, any glass object can be deteriorated (Lovell, 2006). Also it was found that blues, reds, and black tend to be most susceptible to glass disease (Lovell 2006). Additionally, there has been evidence of biological induction in stained glass decay started in the beginning of the 20^{th} century (Krumbein *et al*., 1995).

Fungi produce organic acids that cause etching of glasses subject to acid attack (Jones, 1945). Most previously studies consider that the decay and corrosion of glass plates are related mainly to a physicochemical process (Newton and Davison, 1989; Schreiner, 1991). Furthermore, it has been evidence that the decay could be as a result of biological induction in stained glass decay (Krumbein *et al*., 1995) or by environmental pollution (*i.e.,* CO_2 and SOx) for the stained glass windows, while, in the case of biodeterioration, the presence of organic carbon on the glass (Piñar *et al*. 2013). Micro-organisms like fungi, lichens and algae all favor the maintenance of humidity on the glass surface (Jorba *et al*., 1980). Glass decay includes both chemical and mechanical destruction is caused mainly by microorganisms such as fungi and bacteria (Callot *et al*., 1987; Krumbein *et al*., 1991, 1993; Piñar *et al*. 2013). Additionally, micro-organisms play a considerable part in the degradation of stained glass in cathedrals (Krumbein *et al*. 1991; Jurado *et al*., 2008).

Fungi seem to be able to use CO_2 from the atmosphere to grow (Newton and Davison 1989). X-ray diffraction (XRD) and scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDS) EDAX analysis yields reasonably accurate quantitative results featuring all the elements present in the tested material, namely, C, O, N, Na, S, Al, Si, and Cl (Laskin and Cowin, (2001). The metal oxides added to glass used as colorants and opacities also have an effect on deterioration by fungi (Freestone 2001). The presence of Al_2O_3, Fe_2O_3, and P_2O_5 in the glass can increase its resistance to corrosion or deterioration due to the low solubility of these oxides. It is thought that they immobilize the alkali ions preventing them from moving through the silicate network and being leached out of the glass matrix (Freestone 2001). Paint loss is associated with desiccated surfaces that flakes the paint. This is sometimes caused by excess calcium sulfates on the surface (Griffiths 1989).

Growth of fungi resulted in the dense colonization of the material with an expressed biofilm formation on the glass surface. It was stated that fungi preferred acidic to neutral pH values to develop on glass surfaces and have the ability to metabolize a wide range of C sources (Rodrigues *et al*., 2014). The following deterioration phenomena were observed: micro pitting and crack formation by all studied fungi and cyanobacteria; delineating traces of cells, hyphae and filaments on the glass surface. Crack formation pattern was strain-specific, but appeared to be independent of the chemical composition of the glass itself. The degree of deterioration was changing according to the sensitivity of the glass in question to corrosion (Gorbushina and Palinska, 1999). The pattern of glass biopitting produced in the experiment was very similar to the biopits observed on antique and medieval glasses (Krumbein *et al*., 1991; García-Vallés *et al*., 2003c; Piñar *et al*. 2013).

Stemphylium is one of the most fungal genera listed in the damaged audiovisual collections as well as other documents (Zyska, 1997), magnetic tapes and motion picture films (Czerwińska and Kowalik 1979), and in papyrus samples from different museums in Cairo (Kowalik and Sadurska 1973; Kowalik 1980). Physical and chemical glass surface alterations can be analyzed by means of optical microscopy, Infrared spectroscopy, micro energy dispersive X-Ray Fluorescence, and scanning electron microscopy with energy dispersive spectroscopy analysis (García-Vallés *el al.,* 2003c; Rodrigues *et al*., 2014). The relationship between these mechanisms and a potential role for the extracellular ferric reductase in utilization of environmental and host ferric compounds through the production of free, soluble Fe transferrin both as substrates for the ferric reductase and as iron sources for microorganism growth at neutral pH (Timmerman and Woods, 2001).

The aim of the present work was to use *Stemphylium botryosum* that cause deterioration of stained glasses and comparative study between quantitative compounds of elements present in stained glass without treatment and stained glass infection by common fungi. SEM-EDAX was used to determine the structural changes in the surface, to evaluate the rate of corrosion within the glass, and to determine the composition.

Materials and Methods

Stained Glass Samples:

Six different colored spectrum stained glasses imported from USA namely, black dark purple, opaque white, dark green, black dark, yellow semitransparent and red oxide cuprite were used in the present. The chemical characterization of each glass types, as determined by EDAX, is presented in Table 1, as oxide weight percentages.

A. Black dark purple:

Mn used to get the black color with a Cu, which is no doubt that the manufacturer has succeeded in getting the black color, but it is certain that this was the result of the use of color is pure substances contained a high percentage of Fe (Frank, 1982).

B. Opaque white:

White color caused using calcium (Alumnae) Ca_2 Al_2O_7 (Tite, 2000).

C. Dark green:

The dark green color caused by the presence of Mn (Caley, 1957). Additionally, using of more than oxide to get the green color, and most important of these oxides cupric oxide, which has been used to get the green copper ore and Alstoc Cu-Wollasnite $(Ca,Cu)_3$ Si_3O_9. It has been found that both green and stained glass in green is one of the Chrysocolla ($CuSi_3.nH_2O$) and have found that when cupric oxide entered in the networked installation as a developer, it gives a green color and -may be graded to blue depending on the impurities present as well as the ratio of sodium oxide. It also found that when copper enters the silica in the form of single-valence copper ions, the resulting color green was occurred (Bianchetti *et al*., 2000).

D. Black dark:

Violet color and italics redness produced using Mn_2O_3 Violet/Purple and other colors (Tite, 2000).

Glass color depends upon the result of using of cobalt on the type and installation of glass and the degree of equality of the oxide as the blue color results from the presence of the ion bilateral CO^{+2}, which enters in the networked structure of the glass as a component, where the connection with other ions through four oxygen atoms until this installation up to the case that electrically neutral (Bacci, and Picollo, 1996). Cobalt is used in the form of cobalt aluminate at a high temperature and high alkaline as a tinted glass and it was found that the increase in alkaline ratio increases the puddle color as they reach the proportion of alkali glass between 16% - 21% (Na_2O), while calcium oxide ratio of up to about 4.9% of the total aluminum ratio of about 1.6 to 2% and up in the glass cobalt ratio of about 3 to 5% of glass components (Tite, 2000). It also contains generally more aluminum and less potassium which characterized by this type of cobalt glass from other glass used other colors (Rehren, 2000).

E. Yellow semitransparent:

This color contains sulfur dioxide in a proportion of 0.4% of the weight, Sb_2O_5 to 0.8%, while it reach in opaque glass to about 1.85% and the antimony ratio in the samples could be found from 0.6 to 1.6%(Rehrn, 2000), (Caley, 1957).

F. Red oxide Cuprite:

To make it available to reduced air forces and copper compounds, as well as temperature. It has been found that in the atmosphere oxidant in the presence of a high percentage of iron oxides in the glass with the addition of materials is working to iron mixing in the melt and when cooling the iron oxides are deposited in the form of ferric oxide Fe_2O_3 known as Hematite where given a glass red color tends to brown. Manganese oxides have also been used in coloring glass red because the red color in the case of equilibrium between MnO bilateral and trilateral M_2nO_3.

Colonization of the surface by fungi:

Stained glass samples were cut into two pieces, one to study the fresh fracture and the second for the test pieces. The samples were sterilized using UV light exposure for 48 hours. For the preparation of spore suspensions, 10 mL of sterilized distilled water was added to culture plates containing M40Y (400g sucrose, 20g molt extract, 5g yeast exract and 20g agar) (7-days old), and then spores were freed by the aid of a camel brush on the surface media and put on pieces of glass on the surface of media.

Stained glasses were deliberately inoculated by *Stemphylium botryosum* to study the glass sample before and after infection. The colonization was evaluated after 180 days (Rathore and Chauhan, 2009). SEM model-a FEI Quanta 200 SEM FEG was used to study the colonization of the stained glass surface by *S. botryosum*. It was used to study changes in surface morphology of the deteriorated samples. Glass surfaces were studied by scanning electron microscopy (SEM), attached with energy dispersive spectrometry (EDX), after inoculation for 6 months by *S. botryosum* growth. Macroscopic and microstructural studies (SEM-EDAX) allowed us to identify biodeterioration decay of different stained glass plates in comparison with the untreated glasses (García-Vallés *et al*., 2003c; Piñar *et al*., 2013).

Colormeteric Measurement

Color measurement was investigated by a color difference apparatus (Handy Colorimeter NR-3000, Nippon Denshoku, Tokyo, Japan), calibrated with the standard whiteboard (D65/10, X=82.43, Y=87.40, Z=89.77). Three-dimensional, L*, a* and b* color space by the Commission International de l'Eclairage (CIE) was used for color evaluation. L* specifies the lightness in a range from black (0) to white (100), a* is red-green share and b* is blue-yellow share. Both a* and b* are positive/negative co-ordinates defining the hue and intensity of the color. Color change (ΔE*) was calculated according to equation 1.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where:

L scale: Light vs. dark where a low number (0-50) indicates dark and a high number (51-100) indicates light.

a scale: Red vs. green where a positive number indicates red and a negative number indicates green.

b scale: Yellow vs. blue where a positive number indicates yellow and a negative number indicates blue.

Results and Discussion

Visual Examination

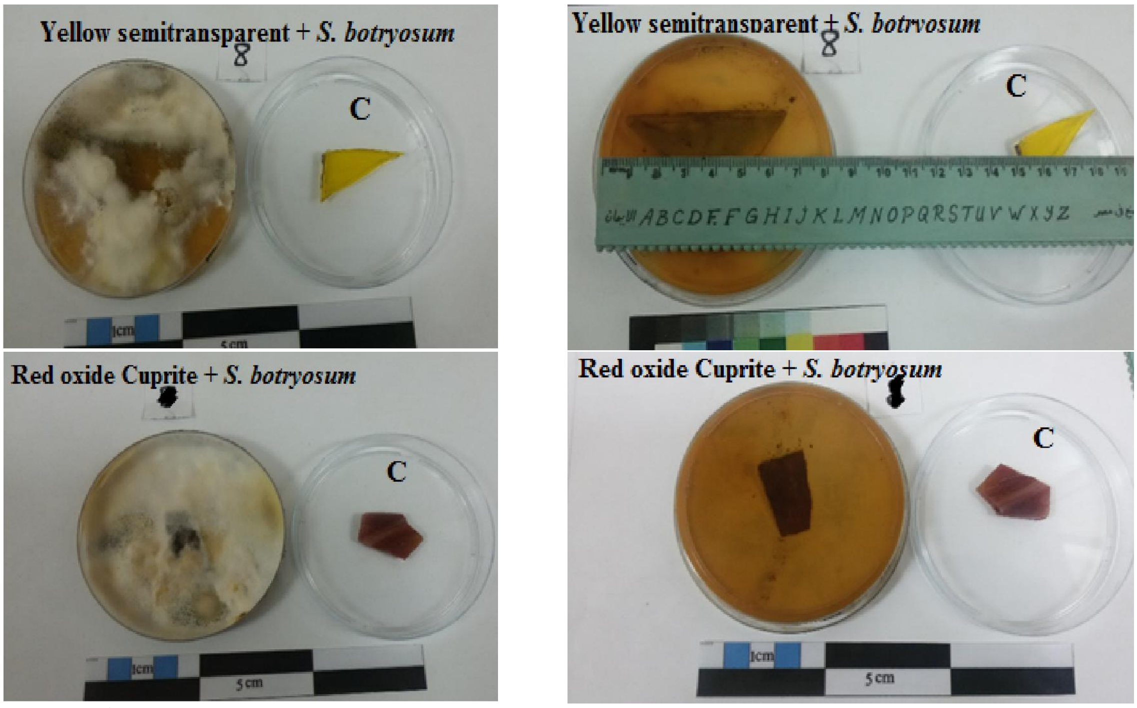
Results showed in Fig. 1 depicts the growth of *S. botryosum* over the surface of the tests glasses. The extent of coverage by *S. botryosum* can be readily discerned. *S. botryosum* growth was extensive on the surface of all test samples, generally covering 80-90% of the surface for all the studied stained glassed.

The previously published studies have focused on the microscopic, chemical and microbial characterization of historical glass samples by culture-dependent techniques (García-Vallés and Vendrell, 2002b; Marvasi *et al*., 2009) or by means of molecular characterization (Rölleke *et al*., 1999; Carmona *et al*., 2006a; Rodrigues *et al*., 2014). But, in the present study, firstly, the visual examination (Fig. 1), with or without magnification, is generally used to identify the decayed glass. Examination of the surface or the interior of the glass can often provide clues as to the nature of the material and whether it is glass. Because glass is an amorphous solid that has melted, if the glass is deteriorated, looking for changes to the surface such as iridescence could help identify the material as glass. Some studied stated that the strong biocorrosive action of fungi on iron-containing olivine resulted in the reduction of glass durability (Callot *et al*., 1987) due to their ability to synthesize ligands specific for ferric iron (Likhtenstein *et al*., 1976).

SEM-EDAX analyses of incubated and unincubated different stained glasses with *Stemphylium botryosum*

Previous studied reported that etching, pit corrosion, and leaching are the initial symptoms of biodeterioration of glass (Krumbein *et al*. 1993). The formation of biomineralization and crusts of the glass is observed due to the redeposition of metals from components of the glass (Jorba *et al*. 1980; Weissmann and Drewello 1996). Also, it was stated that the hyphae penetrated the substrate causing disintegration of its colored and breaking it up by direct mechanical action (Ciferri, 1999). Additionally, the original substrate fragments showed cracks and rough surface containing fungal spores (Milanesi *et al*., 2006).

Petri dish Upper view | Petri dish Lower view



C- Control treatment

Fig. 1: Examination of the colored glasses surface affected by *Stemphlium botryosum.*

EDAX analysis for surface elemental composition of the treated stained glasses with *S. botryosum* is presented in Table 1. The SEM analysis of the incubated and unincubated stained glasses with *S. botryosum* is shown in Fig. 2.

Table 1: Averages of bulk chemical composition of the incubated and unincubated colored glasses with *Stemphylium botryosum*

Glass treatments	Oxides % [a]														
	CO_2	Na_2O	Al_2O_3	SiO_2	P_2O_5	SO_3	Cl_2O	K_2O	CaO	MnO	Fe_2O_3	CoO	CuO	MgO	ZnO	TiO_2
Black dark purple	12.22	7.52	2.90	62.97	-	0.64	0.35	4.39	0.55	0.16	0.55	0.35	0. 41	-	6.99	-
Black dark purple + *S. botryosum*	57.77	4.68	1.30	30.52	-	0.46	0.09	1.73	0.70	0.05	0.16	0.11	0.16	-	2.26	-
Opaque White	11.22	7.81	8.48	62.75	1.31	-	-	3.69	3.89	0.15	0.36	0.12	0.23	-	-	-
Opaque White + *S. botryosum*	46.90	3.52	3.43	41.08	-	0.34	0.13	1.57	2.30	0.19	0.23	0.11	0.20	-	-	-
Dark green	5.73	17.84	2.32	64.13	0.15	0.69	0.25	1.67	6.32	0.11	0.33	0.33	0.15	-	-	-
Dark green+ *S. botryosum*	80.81	0.94	0.66	15.41	0.21	0.46	0.18	0.27	0.72	-	0.14	0.09	0.11	-	-	-
Black dark	8.08	16.21	5.06	60.97	0.20	0.31	0.19	1.16	6.34	0.34	0.59	0.26	0. 30	-	-	-
Black dark purple + *S. botryosum*	67.81	2.33	2.08	25.10	-	0.14	0.04	-	0.34	-	0.16	0.10	0.08	-	0.40	-
Yellow semitransparent	27.01	12.19	2.07	48.85	-	0.11	0.12	1.33	6.52	0.17	0.13	-	0.35	1.17	-	-
Yellow semitransparent + *S. botryosum*	71.49	3.03	0.64	20.90	-	0.30	0.14	0.48	2.36	0.05	0.12	0.12	0.13	0.24	-	-
Red oxide Cuprite	4.11	14.80	3.41	65.40	-	-	-	4.38	1.15	0.20	0.24	0.19	0.18	-	4.56	1.37
Red oxide Cuprite + *S. botryosum*	65.12	3.29	1.34	26.13	-	-	-	1.46	0.38	0.06	0.06	0.11	0.14	-	1.41	0.48

In the black dark purple Mn used to get the black color with Cu, which is no doubt that the manufacturer has succeeded in getting the black color. But it is certain that this was the result of the use of color is pure substances contained a high percentage of Fe in the untreated glass, therefore the treated samples by *S.*

botryosum have been used the elemental peaks of SiO_2 as carbon source (C) was identified as the major composition in black dark purple glass which was decreased from 62.97% to 30.52%. Trace oxides such as Cl_2O, SO_3, Fe_2O_3, CuO, and Ca were also decreased. In the treated glass plate with *S. botryosum*, CO_2 element composition increased to 57.77% compared to the control treatment (12.22%). The major colored elements in the black dark purple glass are Mn, Cu, Fe and Co in the form of oxides) have been used by *S. botryosum* and therefore decreased from (0.16% to 0.05%, 0.41% to 0.16%, 0.55% to 0.16% and 0.35% to 0.11%, respectively). Simultaneously, the EDAX analysis of the treated black dark purple glass with *S. botryosum* indicated the decreases in contents of Na_2O, Al_2O_3, K_2O, and ZnO (Table 1). Furthermore the hyphae of *S. botryosum* were clearly seen,as shown in (Fig. 2A1).

The change in Alumnae composition in the opaque white color glass was from 8.48% (Al_2O_3) in the control sample to 3.43% in the incubated glass with *S. botryosum*. Furthermore, CaO was decreased from 3.89% (control) to 2.30% in the treated sample with *S. botryosum*. It was stated that the fungus has been used the elemental peaks of SiO_2 as C source which was decreased from 62.75% to 41.08% (Table 1). The contents of Na_2O, Al_2O_3, K_2O and CaO were decreased. Small changes were occurred with trace oxides (Fig. 2 B1).

The dark green color caused by the presence of Mn using of more than oxide to get the green color, and most important of these oxides cupric oxide, which has been used to get the green copper ore and Alstoc Cu-Wollasnite $(Ca,Cu)_3\ Si_3O_9$. It has been found that both green and stained glass in green is one of the Chrysocolla ($CuSi_3.nH_2O$) and have found that cupric oxide when he entered in the networked installation as a developer, it gives a green color may be graded to blue depending on the impurities present as well as the ratio of sodium oxide as green turned to blue increasing Na_2O. It also found that when Cu enters the silica in the form of single-valence copper ions Cu In this case, the resulting color green (Fig. 2C1).

For black dark glass, SiO_2 was decreased from 60.97% to 25.10% while CO_2 increased from 8.08% to 67.81%. Trace oxides such as P_2O_5, K_2O, and MnO were completely consumed by the fungus. EDAX analysis of the treated black dark purple glass with *S. botryosum* indicated the decreases in contents of Na_2O, Al_2O_3, CaO, Fe_2O_3 and ZnO (Table 1). Furthermore the hyphae of *S. botryosum* were clearly seen (Fig. 2D1).

In yellow semitransparent glass, SiO_2 was decreased from 48.85% to 20.90% while CO_2 increased from 27.01% to 71.49%. Trace oxides such as CuO and MnO decreased in the treated glasses. EDAX analysis of the treated yellow semitransparent glass with *S. botryosum* indicated the decreases in contents of Na_2O, Al_2O_3, CaO, and MgO (Table 1). Furthermore the hyphae of *S. botryosum* were clearly seen (Fig. 2E1).

SiO_2 was decreased from 65.40% to 26.13% while CO_2 increased from 4.11% to 65.12% in red oxide cuprite glass. EDAX analysis of the treated red oxide cuprite glass with *S. botryosum* indicated the decreases in contents of Na_2O, Al_2O_3, K2O, CaO, ZnO and TiO (Table 1). Furthermore the hyphae of *S. botryosum* were clearly seen (Fig. 2F1).

The percentages of CO_2, K_2O, Na_2O, CaO and SiO_2 were found to be the ones with highest variations in the analyzed samples (Carmona *et al*., 2006b). (García-Vallés *et al*. 2003c) reported that the type of decay in the K-Ca-rich group of glasses resulted in thickness reduction in flat glass. Also, the degree of deterioration changed according to the sensitivity of the glass in question to corrosion (Gorbushina and Palinska, 1999).

We could also observe that the degree of growth or the penetration of *S. botryosum* in the studied stained glasses was related to their chemical composition. Some elements, such as Cu, play a role as inhibitors of the bio-activity (Piñar *et al*., 2013). Additionally, in the red flashed glasses the microbial development was stopped when the organisms reached the Cu-rich colored layer and, thus, they stopped their destructive activity (Piñar *et al*., 2013). Also, it was stated that micro-organisms are able to metabolize Fe and Mn of the glass surface and their intervention in the displacement of such elements is considered (Jorba *et al*., 1980).

Some other works reported that, alumosilicate glasses with network-forming Al_2O_3 and SiO_2, containing a high concentration of Sr and a MgO/Al_2O_3 ratio of 0.3±0.4, were more resistant to molds under tropical conditions (Chebruchan *et al*. 1983), and other one containing Pb and strontium was reported to be successful in bio-corrosion resistance (Shchepochkina 1993). From the present study, *S. botryosum* metabolized iron and manganese oxides (Krumbein 1972; (Jorba *et al*. 1980). Cu oxide also found in all the studied stained glasses and decreased as the glass incubated with *S. botryosum*. Previously, Cu can create different colors in glass, ranging from blue to green. Low lime and high alkali components help to achieve a blue rather than green color with Cu while also destabilizing the glass and rendering it susceptible to deterioration (Weyl 1959; Hancock *et al*. 1994).

The mycelium growth of *S. botryosum* is shown in Fig. 2A1, B1, C1, D1, E1 and F1 for the incubated stained glass plates of black dark purple, opaque white, dark green, black dark, yellow semitransparent and red oxide cuprite, respectively, as compared to the respective control treatment. SEM images show the deterioration of the stained glass plates in the form of distribution of fungal hyphae over the plates. The previous results lead to the fast growth of *S. botryosum* and the formation of the mycelium on the surface of the stained glasses and we have shown in the present study that *S. botryosum* can colonize various types of glass where the chemical elements necessary to its development become available.

Control — Colored glass + *S. botryosum*

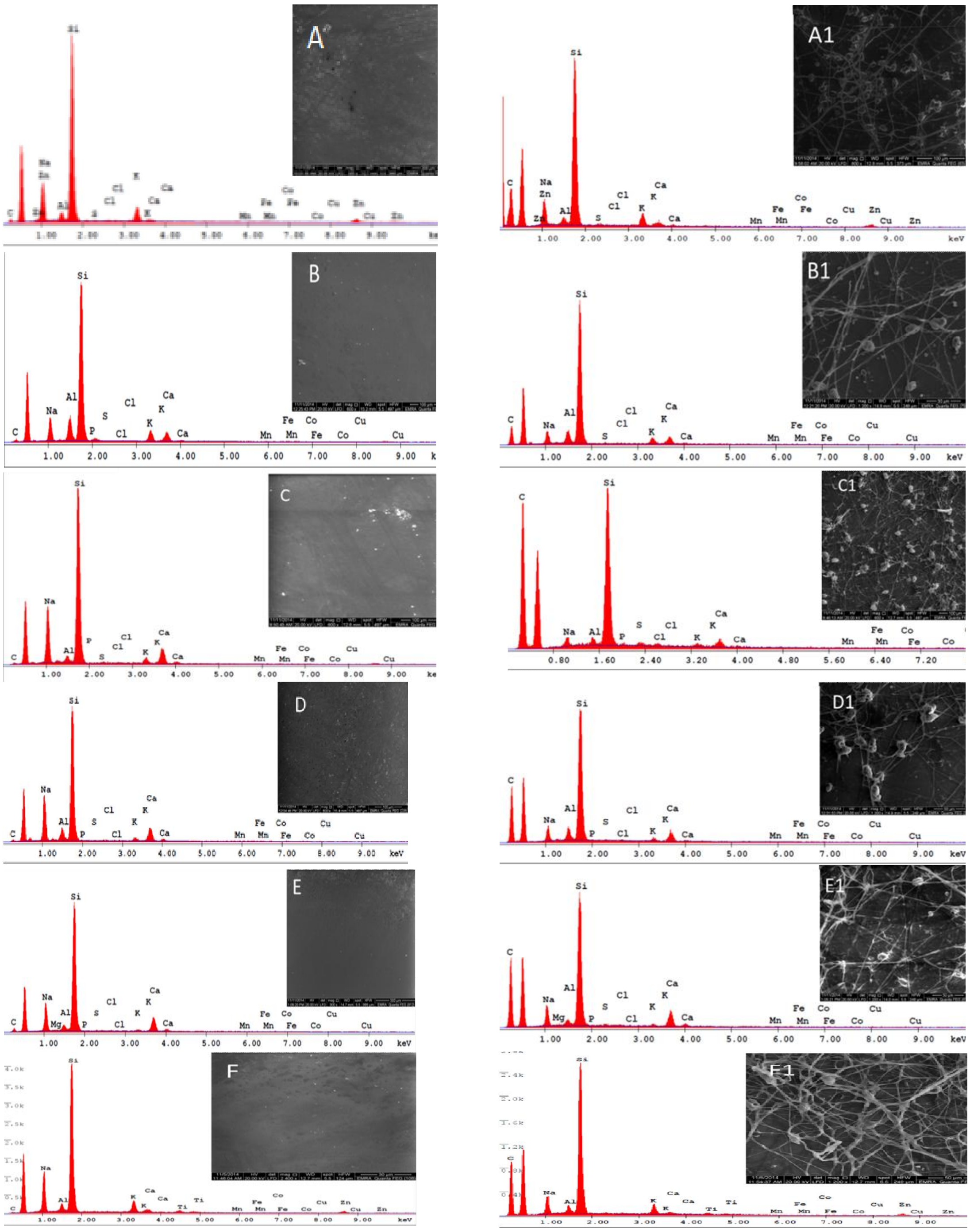


Fig. 2: SEM-EDX images of *S. botryosum* growth on the different colored glasses; A- black dark purple; B- opaque white; C- dark green; D- black dark; E- yellow semitransparent; F- red oxide cuprite.

The deterioration of stained glasses and other materials depends partly on organic compounds and micro-organisms (Crundwell, 2003). The mechanical destruction and leaching environment creation by the adsorption of water, enhancing the chemical destruction of glasses was initiated by the mycelia of filamentous fungi and Actinobacteria (Piñar *et al*. 2013). Furthermore, the produced damages in glass surfaces, such as bio-pitting corrosion, cracks, and patina formation are resulted by the growth of fungi (Krumbein *et al*., 1991; Drewello and Weissmann, 1997). Organic and inorganic acids produced by the growth of fungi, can lead to pH changes, redox-reactions leaching and chelation of special glass components (Piñar *et al*. 2013). Significantly, it was proven that the composition of glass may encourage or retard the microbial activity and, however, the growth-stimulating effect may be enhanced in the presence of Mn as an essential trace metal (Drewello and Weissmann, 1997). Also, significant changes of oxidized Mn and Fe as accumulated salts was shown in the surface layer of glass and could be documented upon a short period of inoculation with fungi (Krawczyk-Bärsch *et al*., 1997). Glass objects with low concentrations of CaO or high concentrations of flux (soda or potash) are susceptible to deterioration, and the larger size of K ions in comparison with Na ions could result in larger holes in the silica network (Karklins ,1983; Hancock, 2013).

Color change measurements

Table 2 presents the values of color changes of the colored glasses as affected by the inoculation of *S. botryosum*. The results showed that from the highest affected glass to lowest one as follows; dark green (ΔE 67.33) > black dark purple (ΔE 61.48) > opaque white (ΔE 28.09) > yellow semitransparent (ΔE 24.43) > red oxide cuprite (ΔE 8.71) > black dark (ΔE 5.33).

Table 2: The chromatic parameters measured for the samples in the L*a*b* (CIE 1976) color system

Treatments	Δ*L*	Δ*a*	Δ*b*	Δ*E**
Black dark purple	10.26	46.74	35.49	61.48
Red oxide Cuprite	7.08	4.74	1.79	8.71
Opaque White	-26.60	2.75	8.60	28.09
Black dark	-1.69	1.36	4.86	5.33
Dark green	19.16	54.21	35.56	67.33
Yellow semitransparent	7.38	22.83	4.62	24.43

Conclusion

The present work has shown, biodeterioration caused by the fungus *S. botryosum* growing on some stained glass plates. Observations evidence of SEM-EDAX revealed the deterioration of the stained glasses. The results obtained in the present study clearly show that *Stemphylium botryosum* able to produce the observed biodeterioration of the investigated stained glasses. It can be concluded that, the changes in oxides composition of the incubated stained glassed with *S. botryosum* suggested that the fungus was used these oxides for growth change of elements as substrates for the reductase , such as iron, Copper manganese and other element sources for fungi growth at neutral, there was a correlation between the level of iron, Copper and manganese transformation and efficacy for these color oxide with an entirely-dependent mechanism of iron, copper and manganese from transformation. Also, in the current state of our work, it is very possible that the consuming of various chemical elements leads in the biomineralization caused by this fungus. The colonization by the fungus was scarcely visible. It is noteworthy that the growth of *S. botryosum* caused some deterioration in stained glass plates and recommended further studies should be done.

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